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HISTOLOGY OF THE PYLORIC CAECA AND ITS CHANGES DURING BROODING AND STARVATION IN A STARFISH, *LEPTASTERIAS HEXACTIS*

FU-SHIANG CHIA

Department of Zoology and Dove Marine Laboratory, University of Newcastle upon Tyne, England

Leptasterias hexactis, the six-rayed starfish, stops feeding completely during more than two months of brooding. Yet, throughout this period, the animal not only spends energy on self-maintenance and care of its young, but it also supports the continuous growth of its oocytes (Chia, 1966, 1968). In other words, the various energy expenditures during brooding have to be derived from nutritional reserves. The cessation of feeding, however, must be considered as behavioral or physiological phenomena because, despite the depletion of nutritional reserves, the animals do not give up brooding and feed, nor are the embryos which are brooded just outside the mouth eaten. It should be recalled that in a closely related species, *Leptasterias groenlandica*, the animals actually brood their young in the cardiac stomach and these are not digested (Lieberkind, 1920; Fisher, 1930).

In considering these questions, a comparative histological study of the pyloric caecum in pre-brooding (feeding), brooding and starved animals is useful, since this is the chief organ for absorption, secretion and storage (see review by Anderson, 1966).

Materials and Methods

Both brooding and pre-brooding animals were collected from Friday Harbor, Washington, during the breeding season (winter months) of 1962. The pyloric caeca were fixed in the following fixatives: Bouin's, Helly's, 4% osmic acid in sea water, and 10% neutral formalin with post-chroming. Materials fixed in osmic acid were embedded in Epon, sectioned at a thickness of one micron on a Porter-Blum ultramicrotome, and stained with Richardson's (Richardson, Jarett, Fink, 1960) stain. This preparation is useful in demonstrating some details of cellular morphology, but it is poor for cytoplasmic inclusions. Materials fixed in other fixatives were embedded in paraffin and sectioned at 5 microns thickness. The combination of Helly's fixative and Mallory's phosphotungstic acid hematoxylin stain (PTAH) gave the best results in demonstrating cytoplasmic inclusions such as the secretory and storage granules. Altman's acid fuchsin and Heidenhain's iron hematoxylin also stained the secretory granules well. Polysaccharide compounds were defined by periodic acid-Schiff's (PAS) reagent. The mercuric bromphenol blue method of Mazia (Mazia, Brewer, Alfert, 1953) was used to demonstrate proteins. Materials fixed in neutral formalin and post-chroming were colored with sudan black to reveal lipid deposits.

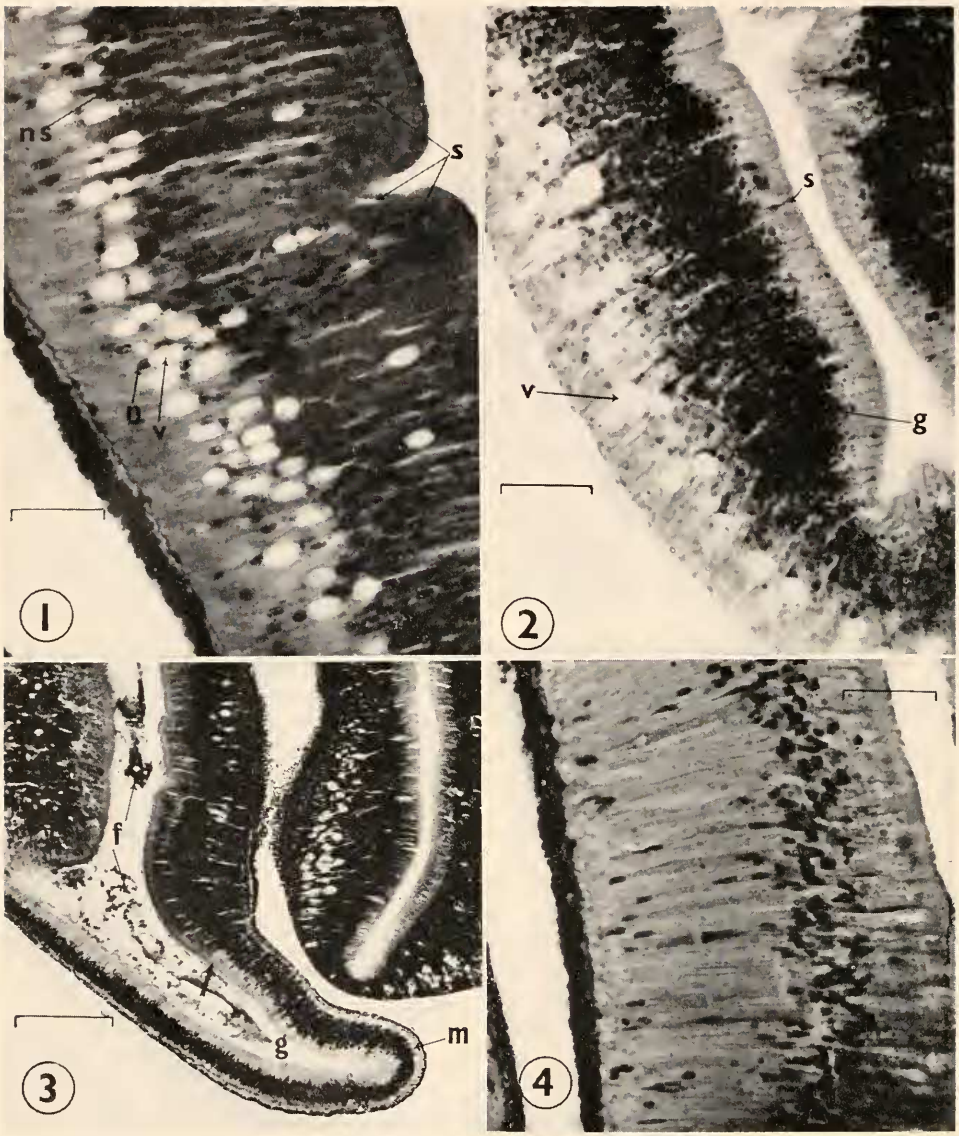

FIGURE 1. Side wall of the pyloric caecum in a feeding animal showing the general histology. Note the relationship of the secretory granules (s), the vacuole (v), the nucleus (n) in the zymogen cell. Note also the nucleus of storage cells (ns). Paraffin. Altman's acid fuchsin-hematoxylin. Scale: 25 μ.

FIGURE 2. Side wall of the pyloric caecum of a feeding animal (mesothelium is not present in this figure), showing the storage granules (g), secretory granules (s) and the vacuole (v). Paraffin. PTAH. Scale: 25 μ.

FIGURE 3. Cross section of the pyloric caecum showing the oral gutter (g) of the median duct where the epithelium proper is composed almost entirely of special current producing cells. Note the thickened middle layer (m) and the coagulated PAS-positive fluid (f) in the lumen

The starvation experiment was carried out at the Friday Harbor Laboratories in the summer months of 1965 and again in 1966. The animals were placed in a small aquarium with circulating sea water at 13–15° C. These animals appeared to be healthy and were able to right themselves even after ten weeks of starvation. Histological sections of the pyloric caeca were prepared from animals at 4-, 8- and 10-week intervals of starvation. A related species, *Leptasterias pusilla,* collected from Shell Beach, northern California, in April, 1966, was also examined. They were subjected to starvation in a recirculating sea water aquarium at 10° C, at Sacramento State College, Sacramento, California. These animals died in the 4th week but apparently not of starvation, as histological sections of the pyloric caecum showed no changes of nutrient reserves in this organ.

General Histology

The general organization and histology of the pyloric caeca in *Leptasterias* corresponds closely to that of *Asterias forbesi* which has been described in detail by Anderson (1953). The general structure is described here only briefly for comparative purposes, except for areas which reveal new or complementary information.

The wall of the pyloric caecum, as in all other starfishes, consists of three layers; an outer mesothelium, a middle layer of connective, muscular and nervous tissues and an inner digestive epithelium (Fig. 1).

The mesothelium or peritoneum is a layer of simple, flagellated, cuboidal epithelium. The thickness of this layer varies depending on the position or the state of contraction. It may be stretched and squamous-shaped, or crowded and low columnar-shaped. As low columnar cells, they measure 5–8 microns tall and a little less in width. The nucleus is oval in shape and it occupies most of the cell.

The middle layer of connective tissue, muscle fiber and nerve plexus again varies in thickness. It is thicker at the floor and roof of the median duct of the pyloric caecum where all three components are clearly shown, yet in other areas it may be so thin that only the nerve plexus and some connective fibers can be detected. The floor of the median duct is evaginated longitudinally to form a definite "gutter" (Fig. 3). The nerve plexus and muscle fibers are highly developed in this area, more so than at any other places in the pyloric caeca.

The digestive epithelium, as in that of *Asterias* (Anderson, 1953), consists of four cell types: (1) storage cells, (2) zymogen cells, (3) special current producers, and (4) mucous cells. All of the cells are cemented by terminal bars at the distal junctions (Fig. 6). In cross sections they all appear irregular or polygonal in shape except the vacuoles in the zymogen cell which are perfectly spherical (Figs. 8, 9).

The storage cells are the major cell type of the digestive epithelium which line all the lumina of the pyloric caeca except the roof and oral gutter of the median duct. The cell measures 50 microns tall at the folds but reaches 120 microns in some other areas and it measures only 2 to 3 microns in diameter. The nuclei are

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of the caecum. Note also the transitions (arrow) between the short special current producers and the tall storage and secretory cells. Paraffin. PAS. Scale: 100 μ.

Figure 4. Side wall of the pyloric caecum of a brooding animal (4 weeks along) showing that the zymogen granules and vacuoles have disappeared. This slide was prepared exactly as the one shown in Figure 1. Scale: 25 μ.

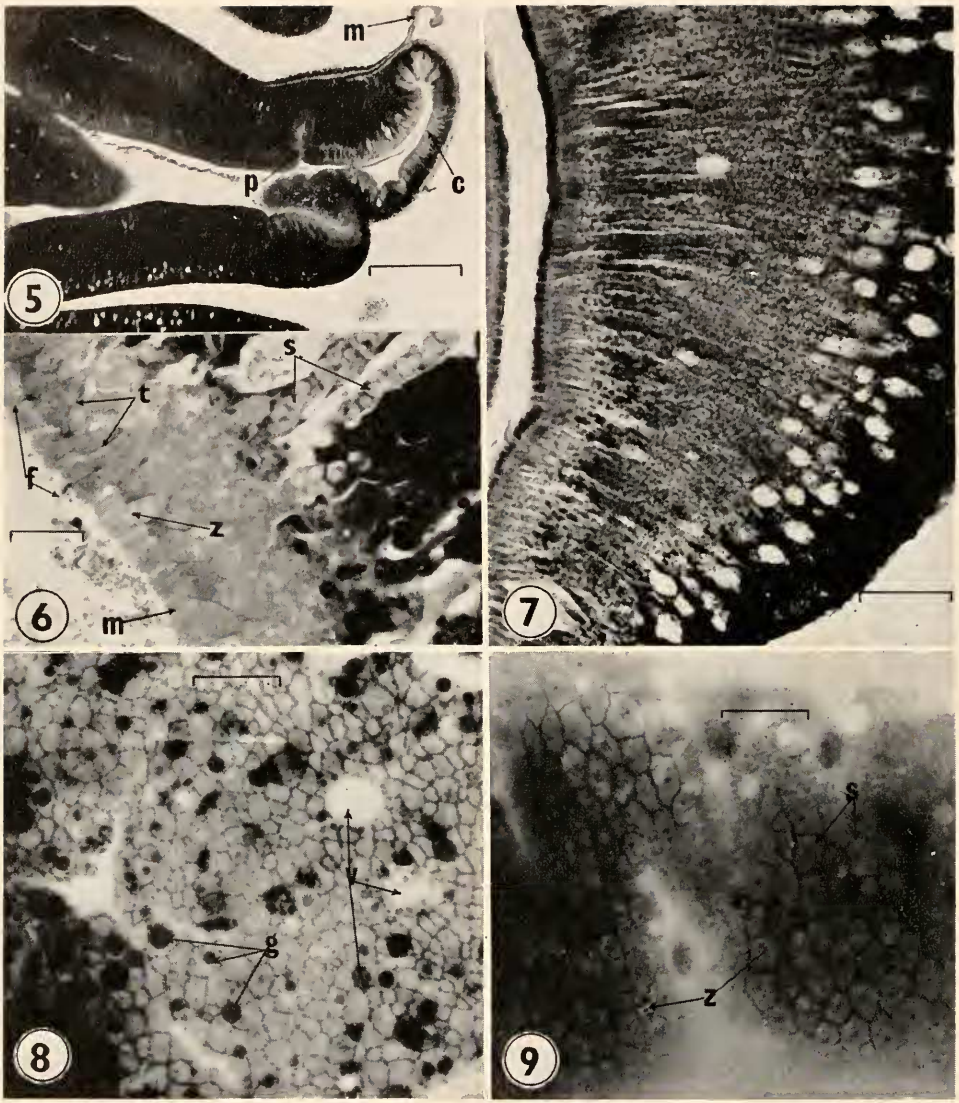

FIGURE 5. Cross section of the pyloric caecum of a feeding animal showing the roof of the median duct between the two mesenteries (m). Note the shorter special current producing cells (c) and the pit (p) in the side wall of storage and secretory area. Paraffin. PAS. Scale: 100 μ.

FIGURE 6. Apical ends of the digestive epithelium of a feeding animal showing the microvilli (m) of the brush border, flagellum (f), terminal bars (t), secretory granules (s), and the free end of the zymogen cell (z). Epon. Richardson's stain. Scale: 5 μ.

FIGURE 7. Side wall of the pyloric caecum of a feeding animal showing the lipid at the basal end of the digestive epithelium. Note also the vacuoles in zymogen cells. Paraffin. Sudan black, wet mount. Scale: 25 μ.

FIGURE 8. Tangential section at the lower middle zone of the digestive epithelium, showing the shape of the epithelial cells in cross section. Note the storage granules (g) in the storage cells and vacuoles (v) in zymogen cells. Paraffin. PTAH. Scale: 10 μ.

elongated and oval in shape and are located in the middle zone of the cells (Fig. 1). The apical ends of the storage cells are provided with a distinct brush border which consists of numerous microvilli (Fig. 6), adding further evidence to support their role as absorptive cells. Each cell also bears a flagellum with its basal body and rootlets centrally located in the apical end of the cell (Figs. 6, 9). This is different from that of *Asterias* in which the basal body is eccentric (Anderson, 1953). The functional significance of this difference is not understood. A number of greenish pigment granules, soluble in formalin, are also present in this region. The ground cytoplasm of the storage cells reacts strongly with PAS reagent except at the apical end where the pigment granules are located (Figs. 3, 5). The PAS reaction is not affected by digestion with diastase. Coarse storage granules are localized at the upper middle zone of the cells (Figs. 2, 8). These granules react positively with PAS, PTAH and mercuric bromphenol blue. The staining behavior thus suggests that the storage granule is a carbohydrate and protein complex. The sudanophilic materials (lipids) are mostly situated at the basal ends of the cells (Fig. 7).

The zymogen cells occur together with the storage cells but are less numerous. They can be identified by the presence of the secretory granules and clear vacuoles (Figs. 1, 2, 7, 8). The cell is flask-shaped with its greatest diameter at the vacuole and lacks the brush border and flagellum at the apical end (Fig. 7). The nuclei are oval or round and are less basophilic than those of the storage cells. They are located at the basal end immediately below the vacuoles (Fig. 1). The secretory granules are well preserved in Helly's fixative and neutral formalin but deformed or clumped together in materials fixed in Bouin's fluid. In most cases, they are arranged in rows between the vacuoles and the apical surface but sometimes they can be observed below the vacuoles or protruding among the brush borders of the adjacent storage cells (Fig. 6).

The special current producers are much shorter than the storage or zymogen cells. They measure 40 to 50 microns in length and line primarily the roof and floor of the median duct (Figs. 3, 5). They have elongated nuclei close to the basal end of the cell and at the apical ends, the brush border and flagella are most prominent. Judging from their highly developed brush borders, it is hard to conceive that they function only as current producers. It is likely that they also serve for absorptive purposes.

A few mucous cells are dispersed among other cells in all parts of the caeca but are more abundant among the current producing cells. The nuclei are round, less basophilic and basally located. The mucous cells, as in zymogen cells, lack both brush border and flagella.

Changes during Brooding and Starvation

The major changes in the pyloric caeca during both brooding and starvation occur primarily in the storage and zymogen cells. Mucous cells and the special current producers do not appear to be affected.

After four weeks of brooding, histological sections show that lipids, and storage

Figure 9. Tangential section at the apical end of the digestive epithelium, showing the storage cells (s) with the centrally located basal bodies of the flagella and the zymogen cells (z) with their secretory granules. Paraffin. PTAH. Scale: 10 μ.

granules, have disappeared from the storage cells. The secretory granules and vacuoles in the zymogen cells have also become inconspicuous (Fig. 4). It is not possible to assess the changes of the nutrient material in the storage cells in quantitative terms, but the changes in zymogen cells can be expressed in a more precise manner because the zymogen cells are identified by their clear vacuoles and during brooding the number of vacuoles decreases. Thus, by comparing the numbers of vacuoles per unit area in the pyloric caeca between feeding and brooding animals, one can get a relatively clear picture. It is estimated that after four weeks of brooding the zymogen cells decrease by about 80%. This does not mean, however, a decline of the cell population; it rather indicates the inactivation of secretory activity. Ten weeks after brooding, which is the end of brooding activity, all observed cellular inclusions in the storage and zymogen cells have been depleted. The ground cytoplasm of the storage cells no longer reacts with PAS reagent. In fact, there are signs of structural breakdown at the apical ends of the digestive epithelium.

In the starved animals there is little or no change in cellular inclusions in both storage and zymogen cells after four weeks of starvation. Even after 10 weeks, there are still some lipids and the storage granules are plentiful. The ground cytoplasm still reacts with PAS reagent although less intensively. In the zymogen cells both the secretory granules and vacuoles are still obvious. This result differs from that of *Asterias* in which all the nutrients are depleted after 8 weeks starvation (Anderson, 1953). Anderson surmised that 8 weeks is about the maximum length of time during which the animal can survive without feeding. *Leptasterias* can apparently survive much longer and the same is true in *Pisaster* which can last as long as 48 weeks without feeding (Mauzey, 1967). This difference in the maximum starvation periods among the three species is likely to be due to the time of year during which the experiments were made; it is well documented that the nutritional level and metabolic rate in the pyloric caeca vary in an inverse relationship with those of the gonad at different seasons of the year (see reviews by Anderson, 1966; Boolootian, 1966). Other factors such as the age and size of the animal may also be important. Finally, there just may be significant differences between species.

Discussion

The most impressive feature of the digestive epithelium is its great height, particularly that of the storage and secretory cells. The tallest cells reach 120 microns with a diameter of only 2 to 3 microns. Thus the ratio between height and diameter is of the order of about 50. Because of the extreme height there is a definite functional zonation or polarity in the cell along its long axis. In the storage cells the nutrient and other cellular inclusions are arranged from the apical end downwards into three bands: pigment granules, storage granules and lipid deposits. In the zymogen cells, the secretory granules most frequently take a position between the vacuole and the apical surface. Because of this relationship, any change of the shape, size or position of the vacuoles would inevitably be connected with the movement of the secretory granules. Therefore, the vacuoles may operate as vehicles to transport the zymogen granules into the lumen of the pyloric caecum.

The highly developed muscular tissue in the oral gutter of the median duct is significant when considering the function of this specialized area. In some particle-feeding starfishes, the whole floor of the median duct is developed into a Tiede-

mann's pouch which has been designated as a "flagellary pumping organ" by Anderson (1960, 1962, 1966). An examination of the floor of Tiedemann's pouch in *Henricia sanguinolenta* has revealed most elaborate circular and longitudinal muscular elements (Fig. 10). There is little doubt that this tissue is directly involved in the pumping mechanism; thus, the transport of nutrient media in the pyloric caeca is accomplished by both flagellary movement and muscular contraction.

The utilization of food reserves during brooding in *Leptasterias* is apparently much greater than during starvation. For example, most of the detectable nutrient in the pyloric caeca is depleted after 4 weeks of brooding, but there are still plenty of nutrients left even after ten weeks of starvation. It is likely that the nutrients in the pyloric caeca can only support the first phase of brooding and at the latter part of brooding, nutrients from other sources, such as the body wall, must be utilized.

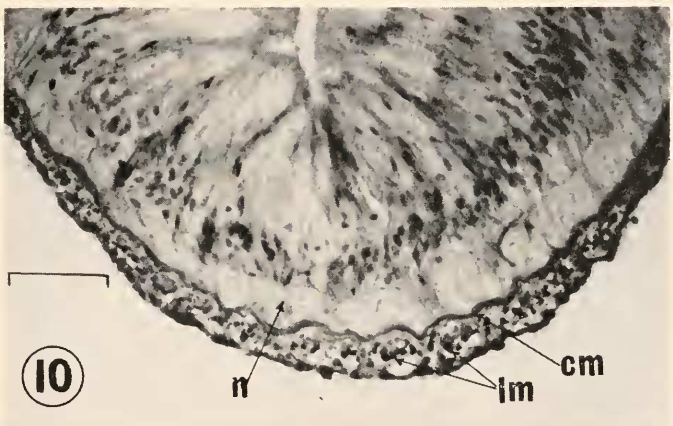

FIGURE 10. Cross section of the oral gutter of Tiedemann's pouch in the starfish, *Henricia sanguinolenta* showing the highly developed nerve plexus (n), the circular (cm) and longitudinal muscles (lm) at the floor of the gutter. Paraffin. PTAH. Scale: 40 μ.

The inactivation of the zymogen cells in the pyloric caeca during brooding provides circumstantial evidence of why *L. hexactis* stops feeding during brooding and how the embryos of *L. groenlandica* survive in the cardiac stomach. It is not certain, however, if the zymogen cells are inactivated after the nutrient has been depleted by starvation. In *Asterias* the depletion of nutrient by starvation has no effect on the zymogen cells (Anderson, 1953), but in *Pisaster* both the nutrient and zymogen granules disappear from the pyloric caeca during the time when the feeding frequency is low and the pyloric caeca are small, or after a long period of starvation (Mauzey, 1966, 1967). As it has been pointed out by Anderson (1966) the so-called zymogen cells may in fact represent several kinds of secretory cells and there is still no direct evidence of the enzymatic nature of the secretions. Wilson and Falkmer (1966) have found insulin-producing cells in the pyloric caeca of *Pisaster* and by using the histochemical methods of Kvistberg (Kvistberg, Lester and Lazarow, 1966) I have identified insulin-producing cells in the pyloric caeca of *Henricia sanguinolenta* (unpublished data). These cells are otherwise inseparable

from zymogen cells, except in *Henricia* where the presumed insulin granules are smaller than the zymogen granules, and most of the insulin-producing cells are located at the lateral diverticular instead of the roof of the median duct where most of the zymogen cells are found. Thus, the pyloric caeca may also function as an endocrine organ.

Summary

1. The general histology of pyloric caeca in *Leptasterias hexactis* is similar to that of *Asterias forbesi,* which is already known.

2. Food reserves such as lipid, polysaccharide and storage granules of carbohydrate-protein complex, are abundant in the pyloric caeca of feeding animals, but disappear after four weeks of brooding. Nutrient reserve from other sources is probably utilized during the last phase of brooding.

3. Accompanying the depletion of nutrient material during brooding, zymogen cells are also inactivated; this is regarded as evidence of why the animals stop to feed during brooding and how the embryos can survive in the cardiac stomach as in the case of *Leptasterias groenlandica.*

4. In *L. hexactis* which have been starved for 10 weeks, the nutrient reserves are still plentiful in the pyloric caeca and, up to this stage, there is little or no detectable change in the zymogen cells.

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